

Attorney Docket No.: 5600.200-US

PATENT

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Sandal et al. Confirmation No: 1759  
Serial No.: 09/426,340 Group Art Unit: 1655  
Filed: October 25, 1999 Examiner: Johannsen, D.

For: Method For Generating A Gene Library

## AMENDMENT UNDER 37 C.F.R. 1.116

Commissioner for Patents  
Washington, DC 20231

Sir:

In response to the Notice of Non-Responsive Amendment, please amend the above-identified application as follows:

## AMENDMENTS TO THE CLAIMS:

Claims 1-19, 21-25 and 27 are pending. Claims 1 and 21 are amended. The following is the status of the claims of the above-captioned application, as amended.

1. (Currently Amended) A method for generating a gene library from an environmental pool of organisms isolated from soil, animal dung, insect dung, insect gut, animal stomach, sea or lake water, waste water, sludge, or sediment, which gene library is enriched in DNA encoding a polypeptide with an activity of interest, which method comprises:

a) subjecting the environmental pool of organisms to cultivation under conditions wherein the pool of organisms is enriched in organisms harbouring said DNA, [wherein said enriched pool of organisms is prepared without screening the organisms for presence of the activity of interest] thereby forming an enriched environmental pool of organisms, and

b) preparing a gene library from the enriched environmental pool of organisms, [wherein said enriched pool of organisms is prepared without screening the organisms for presence of the activity of interest], wherein between step a) and step b) there is no step of isolating organisms having the activity of interest from the enriched environmental pool of organisms.

2. (Previously presented) The method of claim 1, wherein the conditions are culturing in a medium that contains a substrate for the gene product encoded by said DNA.
3. (Previously presented.) The method of claim 2, wherein the substrate constitutes the carbon source and/or nitrogen source of the medium.
4. (Previously presented.) The method of claim 2, wherein the substrate comprises pectin, amylose, cellulose, galactose, xylose or arabinose or a combination thereof.
5. (Previously presented.) The method of claim 1, wherein the pool of organisms is enriched by one or more growth restrictions.
6. (Previously presented.) The method of claim 5, wherein the growth restrictions comprise pH and temperature.
7. (Previously presented.) The method of claim 5, wherein the growth restrictions are pH 9-11 and temperature 50-70°C.
8. (Previously presented.) The method of claim 1, wherein the environmental pool of organisms is isolated from an animal stomach or an insect gut.
9. (Previously presented.) The method of claim 8, wherein the pool of organisms is isolated from a cow's rumen.
10. (Previously presented.) The method of claim 8, wherein the pool of organisms is isolated from the gut of an insect of the *Isoptera*, *Lepidoptera*, *Coleoptera*, or *Diptera* families.
11. (Previously presented.) The method of claim 10, wherein the pool of organisms is isolated from the gut of insects selected from the group consisting of *Agrotis*, *Neotermes castaneus*, *Tineola bisselliella*, and *Melolontha vulgaris*.

12. (Previously presented.) The method of claim 8, wherein prior to isolation, the pool of organisms is enriched by supplying feed to the animal or insect, which comprises a substrate for the polypeptide with an activity of interest.

13. (Previously presented) The method of claim 1, wherein the gene library is enriched in DNA encoding an enzyme of interest.

14. (Previously presented.) The method of claim 13, wherein the enzyme of interest comprises a hydrolase, an oxidoreductase, a transferase, a lyase or a ligase.

15. (Previously presented.) The method of claim 14, wherein the enzyme of interest comprises a protease, lipase, beta-galactosidase, lactase, polygalacturonase, beta-glucosidase, esterase, hemicellulase, peroxidase, oxidase, laccase or glucose oxidase.

16. (Previously presented.) The method of claim 14, wherein the enzyme of interest is a pectinase, an amylase, a galactanase, an arabinase, a xylanase, or a cellulase.

17. (Original.) The method of claim 1, wherein the environmental pool of organisms comprises microorganisms.

18. (Original.) The method of claim 17, wherein the environmental pool of organisms comprises enzyme producing microorganisms.

19. (Previously presented.) The method of claim 17, wherein the microorganisms comprise *Eubacteria*, *Archaeobacteria*, fungi, algae and/or protozoa.

Claim 20 (Cancelled.)

21. (Currently Amended) A method of identifying a DNA sequence encoding a polypeptide of interest from an environmental pool of organisms isolated from soil, animal dung, insect dung, insect gut, animal stomach, sea or lake water, waste water, sludge, or sediment, which method comprises:

- a) subjecting the environmental pool of organisms to cultivation under conditions wherein the pool of organisms is enriched in organisms harbouring said DNA, thereby forming an enriched environmental pool of organisms [wherein said enriched pool of organisms is prepared without screening the organisms for presence of the activity of interest];
- b) producing gene libraries from the enriched environmental pool of organisms [without screening the organisms for presence of the activity of interest], wherein between step a) and step b) there is no step of isolating organisms having the activity of interest from the enriched environmental pool of organisms, and
- c) screening the libraries of step b) to identify a DNA encoding the polypeptide of interest.

22. (Previously presented.) A method of claim 21, wherein the polypeptide of interest encodes an enzyme.

23. (Previously presented.) The method of claim 21, wherein the gene libraries are screened in step c) for an active enzyme.

24. (Previously presented.) The method of claim 21, wherein the polypeptide of interest encodes one of a pectinase, amylase, galactanase, arabinase, xylanase or cellulase.

25. (Previously presented.) A gene library prepared from an environmental pool of organisms enriched in DNA encoding a polypeptide with an activity of interest by the method of claim 1.

Claim 26 (Cancelled.)

27. (Previously presented.) The gene library of claim 25, wherein the polypeptide is an enzyme which comprises a pectinase, an amylase, a galactanase, an arabinase, a xylanase or a cellulase.